Claims

1. A method to prepare a nucleic acid with a nucleotide sequence encoding a modified PKS from a nucleotide sequence encoding a naturally occurring modular PKS wherein said naturally occurring modular PKS contains first regions which encode enzymatic activities and second regions which encode scaffolding amino acid sequences, which method comprises modifying at least one said first region.

2. The method of claim 1 wherein said modifying comprises deleting or inactivating at least one said first region; or

wherein said modifying comprises replacing at least one said first region with a region encoding the corresponding enzymatic activity from a different naturally occurring PKS gene or from a different region of the same naturally occurring PKS gene.

3. The method of claim 1 or 2 wherein said nucleotide sequence encodes at least three PKS modules.

4. The method of any of claims 1-3 wherein said modifying results in utilization of a different extender unit; and/or

wherein said modifying results in utilization of a different starter unit; and/or

wherein said modification results in a polyketide of a different chain length.

5. A nucleic acid comprising a nucleotide sequence encoding a modified PKS obtainable by the method of any of claims 1-4.

6. A cell culture modified to contain the nucleic acid of claim 5.

7. A method to prepare a polyketide which method comprises culturing the cells of claim 6 under conditions wherein said polyketide is produced.

8. A novel polyketide prepared by the method of claim 7.

9. A method to prepare an antibiotic which method comprises glycosylating the polyketide of claim 8.

10. An antibiotic prepared by the method of claim 9.

11. A method to construct a library of colonies containing expression vectors for a multiplicity of different polyketide synthases which method comprises transforming recombinant host cells with a mixture of expression vectors containing the nucleotide sequences obtained by the method of any of claims 1-4; and

separating the transformed cells into individual colonies, and culturing the colonies.

12. A method to prepare a polyketide combinatorial library which method comprises culturing the library of colonies obtained by the method of claim 11 under conditions wherein said polyketides are produced.

13. A multiplicity of cell colonies comprising a library of colonies wherein each colony of the library contains an expression vector comprising a nucleotide sequence encoding a modular PKS derived from a naturally occurring PKS gene cluster wherein at least one enzymatic activity has been deleted and/or replaced by a different version of said activity or is mutated so as to result in a polyketide other than that produced by said naturally occurring PKS and

wherein the nucleotide sequence contained in each colony in the library encodes a different PKS.

14. The multiplicity of cell colonies of claim 13 wherein in said library of colonies said naturally occurring PKS gene cluster is the erythromycin gene cluster; and/or

wherein, in at least one colony of said library, said different version is the corresponding enzymatic activity from a different modular PKS or from another location in the same PKS gene cluster; and/or

wherein the number of PKS modules contained in the expression vector is different in at least two colonies of the library; and/or

wherein the extender unit utilized by the encoded PKS is different in at least two colonies of said library; and/or

wherein the starter unit utilized by the enclosed PKS is different in at least two colonies of said library; and/or

wherein the reduction cycle specificities are different in at least two colonies of said library.

15. A method to produce a library of modular PKS proteins which method comprises culturing the multiplicity of cell colonies or the library of colonies of claim 13 or 14 under conditions wherein said expression vectors effect production of said modular PKS proteins.

16. A library of PKS proteins prepared by the method of claim 15.

17. A multiplicity of cell colonies comprising a library of colonies wherein each colony of the library contains a modular PKS derived from a naturally occurring PKS wherein at least one enzymatic activity has been deleted or replaced by a different version of said activity or is produced from a mutated form of said gene so as to result in a polyketide other than that produced by said naturally occurring PKS, and

each colony in the library contains a different PKS.

18. The multiplicity of cell colonies of claim 17 wherein said naturally occurring PKS is the erythromycin PKS; and/or

wherein the number of modules of PKS is different in at least two colonies of the library; and/or

wherein the extender unit utilized by the PKS is different in at least two colonies of the library; and/or

wherein the starter unit utilized by the PKS is different in at least two colonies of the library; and/or

wherein the reduction cycle specificities are different in at least two colonies of said library.

19. A method to produce a combinatorial library of polyketides which method comprises culturing the cell colonies or library of colonies of claim 17 or 18 under conditions wherein polyketides whose synthesis is effected by said different PKS proteins are produced.

20. A combinatorial library of polyketides prepared by the method of claim 19.

21. A multiplicity of polyketides which comprises a combinatorial library of polyketides which results from culturing colonies containing polyketide synthases derived from a naturally occurring PKS wherein at least one enzymatic activity has been deleted and/or replaced by a different version of said activity or is mutated so as to result in a polyketide other than that produced by said naturally occurring PKS, wherein each PKS in said library produces a different polyketide.

22. The library of claim 21 wherein the chain length is different in at least two polyketides; and/or

which contains at least two polyketides formed from different extender units; and/or

which contains at least two polyketides of different oxidation states; and/or

which contains at least two polyketides of differing stereochemistry; and/or

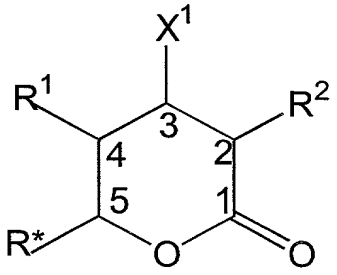
which contains at least two polyketides formed from different starter units.

23. A method to identify a successful candidate polyketide which binds to or reacts with a target moiety, which method comprises screening the library of claim

20, 21 or 22 by contacting each polyketide in said library with the target moiety under conditions wherein a successful candidate would form a complex with said target moiety, and

detecting any complex formed, thus identifying a polyketide of the library as the successful candidate.

24. A compound of the formula:



(1)

including the glycosylated and isolated stereoisomeric forms thereof;

wherein R* is a straight chain, branched or cyclic, saturated or unsaturated substituted or unsubstituted hydrocarbyl of 1-15C;

each of R^1 and R^2 is independently H or alkyl (1-4C) wherein any alkyl at R^1 may optionally be substituted;

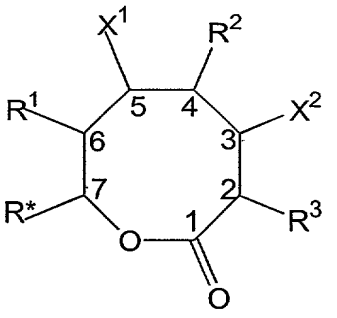
X^1 is H_2, HOH or =O;

with the provisos that:

at least one of R^1 and R^2 must be alkyl (1-4C); and

the compound is other than compounds 1, 2, 3, 5 and 6 of Figure 6A;

or of the formula:



(2)

including the glycosylated and isolated stereoisomeric forms thereof;

wherein R* is a straight chain, branched or cyclic, saturated or unsaturated substituted or unsubstituted hydrocarbyl of 1-8C;

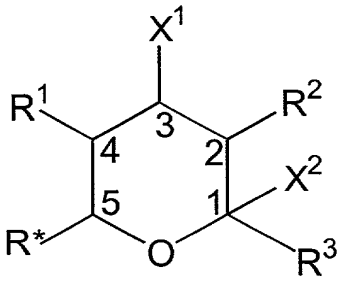
each of R^1, R^2 and R^3 is independently H or alkyl (1-4C) wherein any alkyl at R^1 may optionally be substituted;

each of X^1 and X^2 is independently H_2, HOH or =O;

with the proviso that:

at least two of R^1, R^2 and R^3 are alkyl (1-4C);

or of the formula:



(3)

including the glycosylated and isolated stereoisomeric forms thereof;

wherein R* is a straight chain, branched or cyclic, saturated or unsaturated substituted or unsubstituted hydrocarbyl of 1-15C;

each of R^1, R^2 and R^3 is independently H or alkyl (1-4C) wherein any alkyl at R^1 may optionally be substituted;

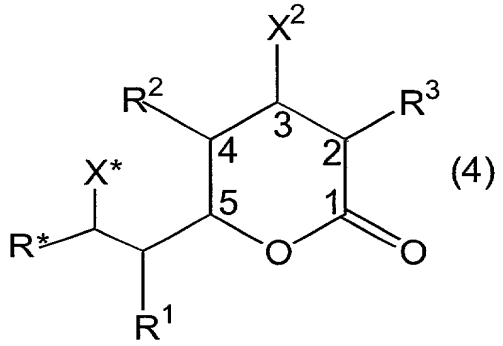
each of X^1, X^2 and X^3 is independently H_2, HOH or =O;

with the provisos that:

at least one of R^1 and R^2 must be alkyl (1-4C); and

the compound is other than compound 8 of Figure 6A;

or of the formula:



(4)

including the glycosylated and isolated stereoisomeric forms thereof;

wherein R* is a straight chain, branched or cyclic, saturated or unsaturated substituted or unsubstituted hydrocarbyl of 1-8C;

each of R^1, R^2 and R^3 is independently H or alkyl (1-4C) wherein any alkyl at R^1 may optionally be substituted;

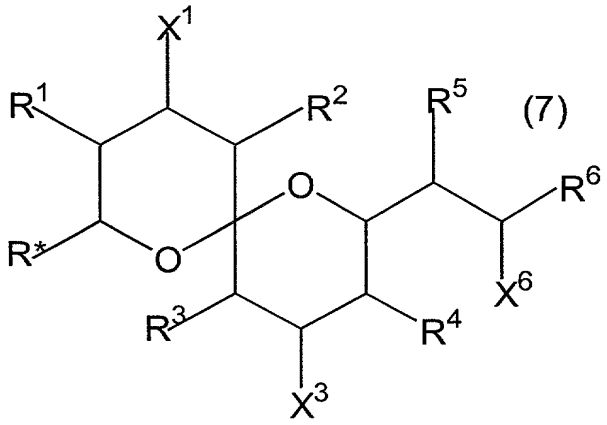
each of X* and X^2 is independently H_2, HOH or =O;

with the proviso that:

at least one of R^2 and R^3 is alkyl (1-4C); and

the compound is other than compound 9 of Figure 6A;

or of the formula:



including the glycosylated and isolated stereoisomeric forms thereof;

wherein R* is a straight chain, branched or cyclic, saturated or unsaturated substituted or unsubstituted hydrocarbyl of 1-15C;

each of R^1-R^5 is independently H or alkyl (1-4C) wherein any alkyl at R^1 may optionally be substituted;

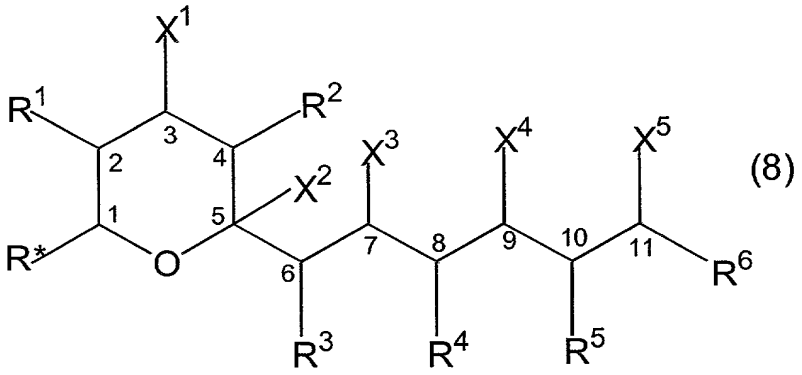
R^6 is alkyl (1-5C);

each of X^1 and X^3 and X^6 is independently H_2, HOH or =O;

with the proviso that:

at least two of R^1-R^4 are alkyl (1-4C);

or of the formula:



(8)

including the glycoslated and isolated stereoisomeric forms thereof;

wherein R* is a straight chain, branched or cyclic, saturated or unsaturated substituted or unsubstituted hydrocarbyl of 1-15C;

each of R^1-R^5 is independently H or alkyl (1-4C) wherein any alkyl at R^1 may optionally be substituted;

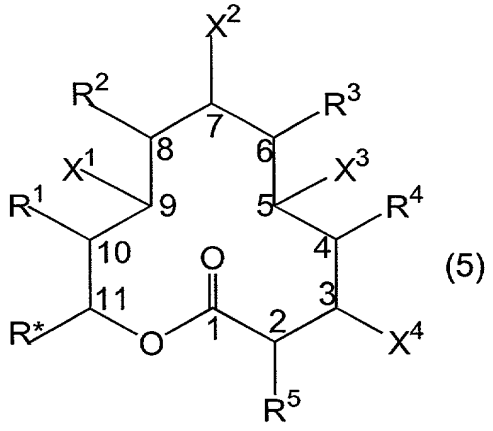
R^6 is alkyl (1-5C);

X^2 is OH or H;

each X^1, X^3, X^4 and X^5 is independently H_2, HOH or =O; or X^4 is H and the compound of formula (8) has a π-bond between positions 9-10, with the proviso that:

if X^2 is H, at least one of X^3 and X^4 is HOH or =O.

25. A compound of the formula:



(5)

including the glycosylated and isolated stereoisomeric forms thereof;

wherein R* is a straight chain, branched or cyclic, saturated or unsaturated substituted or unsubstituted hydrocarbyl of 1-15C;

each of R^1, R^2, R^3, R^4 and R^5 is independently H or alkyl (1-4C) wherein any alkyl at R^1 may optionally be substituted;

each of X^1, X^2, X^3 and X^4 is independently H_2, HOH or =O; or

X^1 or X^2 or X^3 or X^4 is H and the compound of formula (5) contains a π-bond at positions 8-9 or 6-7 or 4-5 or 2-3;

with the proviso that:

at least two of R^1-R^5 are alkyl (1-4C); and

the compound is other than compound 13 or 14 of Figure 6A or compound 205, 210-213 of Figure 11.

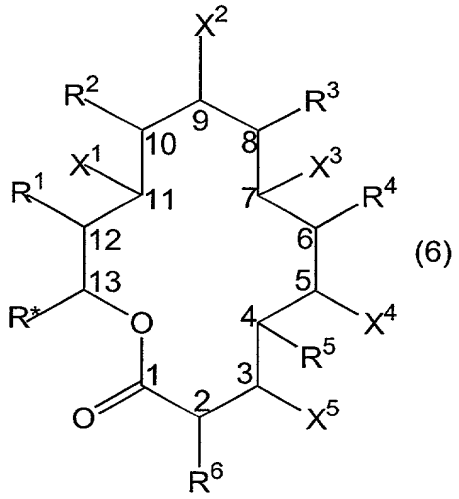
26. The compound of claim 25 wherein at least three of R^1-R^6 are alkyl (1-4C); and/or

wherein X^1 is -OH; and/or

X^2 is =O; and/or

X^3 is H.

27. A compound of the formula:



(6)

including the glycosylated and isolated stereoisomeric forms thereof;

wherein R* is a straight chain, branched or cyclic, saturated or unsaturated substituted or unsubstituted hydrocarbyl of 1-15C;

each of R^1-R^6 is independently H or alkyl (1-4C) wherein any alkyl at R^1 may optionally be substituted;

each of X^1-X^5 is independently H_2, HOH or =O; or

each of X^1-X^4 is independently H and the compound of formula (5) contains a π-bond in the ring adjacent to the position of said X at 2-3, 4-5, 6-7, 8-9 and/or 10-11;

with the proviso that:

at least two of R^1-R^6 are alkyl (1-4C); and

the compound is other than compounds 17, 24 or 28 of Figure 6B, compound 301-311 of Figure 12(A) or compound 312-322 of Figure 12(B).

28. The compound of claim 27 wherein at least three of R^1-R^6 are alkyl; and/or

X^2 is =O; and/or

X^1 is OH; and/or

X^4 and X^5 are OH; and/or

R^* is substituted alkyl and/or

R^1 is substituted alkyl.